Date of Approval: March 11, 2016

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-392

IMRESTOR

pegbovigrastim injection

Periparturient Dairy Cows and Periparturient Replacement Dairy Heifers

"For the reduction in the incidence of clinical mastitis in the first 30 days of lactation in periparturient dairy cows and periparturient replacement dairy heifers"

Sponsored by:

Elanco Animal Health,
A Division of Eli Lilly & Co.

Table of Contents

I. GENERAL INFORMATION

A. File Number

NADA 141-392

B. Sponsor

Elanco Animal Health,
A Division of Eli Lilly & Co.
Lilly Corporate Center
Indianapolis, IN 46285

Drug Labeler Code: 000986

C. Proprietary Name

IMRESTOR

D. Established Name

Pegbovigrastim injection

E. Pharmacological Category

Immunomodulator, bovine granulocyte stimulating factor

F. Dosage Form

Injectable solution

G. Amount of Active Ingredient

15 mg pegbovigrastim per 2.7 mL syringe

H. How Supplied

Pre-filled single-dose syringe

I. Dispensing Status

Rx

J. Dosage Regimen

Administer the first dose (syringe) 7 days prior to the cow's or heifer's anticipated calving date. If necessary, the first dose may be administered within a range of 4 to 10 days prior to the anticipated calving date to accommodate management schedules. Administer the second dose (syringe) within 24 hours after calving.

K. Route of Administration

Subcutaneous injection

L. Species/Class

Periparturient dairy cows and periparturient replacement dairy heifers

M. Indication

For the reduction in the incidence of clinical mastitis in the first 30 days of lactation in periparturient dairy cows and periparturient replacement dairy heifers.

II. EFFECTIVENESS

A. Dosage Characterization

Two pilot field studies were conducted at a commercial dairy in the U.S. to evaluate the effectiveness and animal safety of various dose levels of pegbovigrastim injection against naturally occurring clinical mastitis in periparturient multiparous dairy cows with pre-screening lymphocyte counts < 15,000 cells/µL and no abnormal health conditions. In each study, there were approximately 50 cows in each treatment group that completed the study (i.e., cows that received both doses and were not removed from the data set for non-study related reasons). Animals were evaluated for the incidence of clinical mastitis, based on the clinical appearance of the udder and milk for the first 30 days post-calving. Safety parameters included milk composition and quality, daily milk production, percent live births, the number of days between the first dose and calving, first service conception rates, and calf health. Blood was collected for absolute neutrophil counts prior to the Day -7 dose, approximately 24 hours after the first dose (Day -6), and on Day 7 post-calving.

The first study evaluated 20 and 40 µg pegbovigrastim injection/kg body weight (BW) administered by subcutaneous injection 7 days prior to the anticipated calving date and again within 24 hours after calving. A negative control group (saline) and a positive control group (non-PEGylated bovine granulocyte colony stimulating factor) were included in the study. Absolute neutrophil counts were elevated at 24 hours after the first dose, and remained elevated through Day 7 post-calving in both pegbovigrastim injection groups compared with saline. Clinical mastitis incidence rates of 8% (40 µg/kg BW), 12% (20 µg/kg BW), 25% (positive control), and 44% (saline) were observed. Cows in the 40 µg/kg BW group had reduced milk production throughout the study; otherwise there were no biologically relevant changes in the safety parameters.

The second study evaluated 5, 10, and 20 µg pegbovigrastim injection/kg BW administered by subcutaneous injection 7 days prior to the anticipated calving date and again within 24 hours after calving. A negative control group (saline) was included in the study. Absolute neutrophil counts were elevated in all pegbovigrastim injection groups compared with saline at 24 hours after the first dose, and remained elevated through Day 7 post-calving in the 10 µg/kg BW and 20 µg/kg BW groups. Clinical mastitis incidence rates of 9% (20 µg/kg BW), 17% (10 µg/kg BW), 20% (5 µg/kg BW), or 34% (saline) were observed. There were no biologically relevant changes in the safety parameters.

Based on the reduced incidence of mastitis seen in these studies, 20 µg/kg BW was selected for evaluation in the field studies. The concentration of active ingredient in the pre-filled syringes (15 mg per 3 mL) was set to achieve this dose

when administered to a 750 kg cow (the approximate weight of a pregnant mature Holstein).

B. Substantial Evidence

Dose Confirmation Study

1. Title: "Effectiveness and Clinical Safety of PEG bG-CSF against Naturally Occurring Clinical and Subclinical Mastitis in Periparturient Cows and Heifers". Study numbers 2011-01-03-01-032, -033, -034, and -035; and 2011-01-03-02-037. January 2011 to December 2011.

2. Study Numbers and Locations:

Table II.B.1. Study Numbers and Locations

	Study Number	**Study Location**
WI	2011-01-03-01-032	Lodi, WI
WA	2011-01-03-01-033	Granger, WA
CO	2011-01-03-01-034	Platteville, CO
CA	2011-01-03-01-035	Hanford, CA
FR	2011-01-03-02-037	Sombrin, France

3. Study Design:

   a. *Objective:* To evaluate the effectiveness and clinical safety of pegbovigrastim injection (PEGylated bovine granulocyte colony stimulating factor) against naturally-occurring clinical mastitis in periparturient dairy cows and heifers under field conditions.

   b. *Study Animals:* Healthy periparturient (estimated to be approximately 7 days prior to calving) crossbred and/or purebred cows or heifers, including small frame (Jerseys) and large frame (Holstein and Holstein-cross) breeds, were enrolled in the study. Animals were part of a commercial dairy herd at each study site. A total of 243 heifers and 558 cows (801 animals total) were enrolled across the four U.S. sites and the France site. Enrolled heifers and cows, and calves born to treated dams, were identified using unique individually numbered ear tags and colored leg bands. Animals were housed at the commercial dairy and managed according to typical husbandry practices.

   c. *Experimental Design:* Within each site, treatments (pegbovigrastim injection or saline) were randomly assigned to animals as they qualified for enrollment. The individual animal was the experimental unit. All study site personnel except the treatment administrators, who did not participate in subjective evaluations, were masked to treatment assignments.

   d. *Test Article Administration:* The test article was pegbovigrastim injection, packaged in 3 mL pre-filled ready-to-use plastic syringes containing 15 mg active ingredient. The negative control article was sterile saline (0.9% NaCl), packaged in 3 mL pre-filled ready-to-use plastic syringes. Animals were administered either pegbovigrastim injection or saline by SC injection

of the contents of one syringe in the neck when they were identified as being approximately 7 days before their anticipated calving date (Day -7), and again within 24 hours after calving (called Day 0, regardless of calving date). At each site, 80 animals (or 81 saline-treated animals at one site) were assigned to each treatment group.

e. *Measurements and Observations:* Enrolled animals were observed twice daily for 7 days following each injection for general health and adverse reactions (including injection site reactions). Animals were observed once daily from Days 8 to 30 for general health observations. Physical examinations were conducted on enrolled animals on Days -7 and 0, and on calves born to study cows on Days 0 and 30. Cow weights were determined via weight tape on Day -7. Calf weights were determined using calibrated scales on Day 0 and Day 30.

Each quarter of each enrolled animal was evaluated at each milking from Days 3 to 30 to monitor the development of clinical mastitis. Clinical scores were assigned for each quarter as follows:

1 = normal quarter/normal milk (no California Mastitis Test [CMT] test performed)
2 = normal quarter/suspect milk (a few/transient clots or flakes, or slight discoloration) and a CMT score of negative, trace, or 1
3 = normal quarter/abnormal milk (flakes, clots, or discoloration) OR abnormal quarter (slight inflammation)/normal milk, and a CMT score ≥ 2
4 = abnormal quarter (moderate inflammation) /abnormal milk (obvious flakes, clots, or discoloration; bloody or serous) and a CMT score ≥ 2
5 = abnormal quarter (severe inflammation) /abnormal milk (numerous flakes, clots, or severe discoloration; severe blood or serous consistency) and a CMT score ≥ 2

Animals with clinical scores ≥ 3 had their rectal temperature taken. Animals with systemic clinical signs (inappetence, depression, and either abnormally low [<100 °F] or abnormally high [≥ 104 °F] temperatures) and cows that are agalactic were scored a 5. Duplicate individual quarter milk samples were collected for microbiological analysis from animals with a clinical score ≥ 3.

On Days 27, 28, and 30, a CMT was performed on each quarter with clinical scores 1 or 2 to determine the presence of subclinical mastitis. Duplicate individual quarter milk samples were collected for microbiological analysis. Composite milk samples were collected for milk composition (somatic cell counts [SCC], milk fat, milk protein, milk lactose, and milk solids) from all milkable quarters at one milking on Days 7, 14, 21, and 28. Milk production was recorded daily from Days 1 to 30.

Reproductive parameters assessed were first service conception rates (as determined by rectal palpation or ultrasound approximately 30 to 40 days post-artificial insemination), percent live births, calf mortality and health observations, and length of gestation. Other parameters assessed were

injection site reactions, cow mortality and health observations for clinical safety, and absolute leukocyte counts for drug exposure confirmation.

First service conception rates (determined using rectal palpation or ultrasound approximately 30 to 40 days post-calving) and calf health observations (Days 0 to 30) were also collected. Animals that died or were euthanized during the study were necropsied by a veterinarian.

4. Statistical Analysis: The primary variable was the incidence of clinical mastitis. An animal was identified as a clinical mastitis treatment failure if one or more quarters were scored a clinical score ≥ 3 at any milking from Days 3 to 30. Subclinical mastitis, milk composition, milk production, gestation length, percent live births, calf health observations, and first service conception rates were evaluated as safety variables. Absolute leukocyte counts were evaluated to confirm exposure to the test article. Microbiological results were summarized for each treatment group.

   The effectiveness analysis was conducted using the combined U.S. and France sites. A generalized mixed model for binomial data was used to compare the control group clinical mastitis incidence rates (failure rates) to the pegbovigrastim injection group failure rates, including treatment as a fixed effect, and site and site by treatment as random effects. A two-sided test with a 0.05 level of significance was used for the effectiveness analysis.

   Clinical safety analyses were conducted using the combined U.S. and France sites. Generalized mixed models were used where the data distribution was selected to correspond to the type of variable, i.e., the binomial distribution for percent data and the normal distribution for continuous data. Two-sided tests with a 0.10 level of significance were used for the statistical analysis of clinical safety endpoints.

5. Results: Animals were removed from the study for humane reasons requiring therapeutic intervention (debilitating or irresolvable disease, condition, or injury), or if they had dystocia or other abnormal calving events that were reasonably expected to confound the study. Animals removed from the study for non-mastitis related reasons prior to the completion of Day 30 were excluded from the effectiveness data set. Additional animals were excluded from the effectiveness data set for significant protocol deviations. A total of 69 pegbovigrastim injection-treated cows and 63 saline-treated cows were excluded from the effectiveness data set across all five sites.

   a. *Clinical Mastitis:* Administration of pegbovigrastim injection resulted in a statistically significant difference ($p = 0.025$) in the incidence of clinical mastitis (treatment failure rate) across all five sites with the difference in favor of the pegbovigrastim injection-treated group (failure rate: 60/331 = 18.13%) compared to the saline-treated group (failure rate: 85/338 = 25.15%).

   b. *Clinical Safety:*

      1) Injection Site Reactions: There were no injection site reactions observed after administration of the test article in this study.

2) Absolute Leukocyte Counts at Days -7, -6, and 7 relative to parturition: In general, animals receiving pegbovigrastim showed a mild transient increase in the absolute number of circulating neutrophils. A mild decrease in the lymphocyte percentage was also seen in the treated group, although not in all treated animals. The absolute number of lymphocytes was not affected when there was a decrease in the lymphocyte percentage.

c. *Milk Composition and Production:* Administration of pegbovigrastim injection had no clinically relevant impact on milk composition (SCC, milk fat, milk protein, milk lactose, and milk solids) or milk production.

Daily milk production means for each treatment group were compared for large and small frame animals separately. Large frame animals included Holsteins or Holstein-crosses that were used at four sites (CA, CO, WA, France). Small framed animals included Jerseys that were used at one site (WI). There was no statistically significant difference in milk production between treated and control groups for the large frame breeds ($p = 0.2958$) and the small frame breeds ($p = 0.4685$).

6. Adverse Events: There were no adverse events in cows or calves that were associated with administration of pegbovigrastim injection.

7. Conclusion: This study demonstrates that pegbovigrastim injection administered approximately 7 days prior to the anticipated calving date and again within 24 hours after calving is effective for reducing the incidence of clinical mastitis in the first 30 days of lactation in periparturient dairy cows and periparturient replacement dairy heifers.

III. TARGET ANIMAL SAFETY

The clinical effects of pegbovigrastim injection administered via subcutaneous injection were evaluated in two target animal safety studies. In the first margin of safety study pegbovigrastim was administered to periparturient Jersey cows and heifers at 1X, 2X, and 3X (15 mg, 30 mg, and 45 mg per animal in pre-filled syringes) the recommended therapeutic level of 15 mg pegbovigrastim for a total of three injections: days -7 and -3 relative to expected parturition, and within 24 hours after parturition. The results of this study showed an increase in abomasal ulcers in the treated animals. Abomasal ulcers are thought to occur as a result of physiologic stress or direct pathology to the abomasum. Periparturient dairy cows naturally undergo considerable physiologic stress during the transition period between non-lactation and the extreme metabolic demands of high milk production immediately post calving. It is hypothesized that during this period, an animal is more likely to develop abomasal ulcerations; however, incidence rates on this condition in the sub-population of periparturient dairy cows is not known.

A second study was conducted to see if the results from the first study were reproducible and to more closely study the pathology of the abomasal ulcers/gastrointestinal pathology. In the second margin of safety study pegbovigrastim was administered to periparturient Holstein cows at 1X, 2X, 2.5X, and 3X the recommended therapeutic level of 15 mg pegbovigrastim for a total of two injections: day -7 relative to expected parturition and within 24 hours after parturition. This change in dosage administration was made because the purpose of

the second margin of safety study was primarily to focus on gastrointestinal pathology under the proposed conditions of use. The design of the second margin of safety study was altered to decrease environmental stress in the study animals which could have been a contributing factor in ulcer formation in the first study. In the second study, animals were kept at their home location instead of being re-located to a study site. Additionally, the diets fed in the second study were not changed, as they were in the first study, and cows were kept in larger stalls to increase cow comfort while still being individually housed. These conditions more closely matched husbandry practice of commercial U.S. dairy farms. Necropsies were conducted at a later time point in order to assess whether ulcers, if present, were healing. Additionally, a more uniform population of study animals was used.

An injection site safety study was conducted to evaluate the injection site toleration of non-lactating cows treated subcutaneously with the contents of one syringe (15 mg) pegbovigrastim at 12 hours and 14 days after administration.

Reproductive safety as it pertained to late gestation, neonatal calf health, and first service conception rate, was evaluated as part of the field effectiveness study. It was determined that reproductive safety for the first and second trimesters was not required for this approval given the product is not intended to be used during these times in the target animal. The margin of safety studies, the injection site safety study, and the reproductive safety study are summarized below.

A. First Margin of Safety Study

1. Title: "Target Animal Safety in Dairy Cows Injected Subcutaneously with PEG bG-CSF at 1X, 2X, and 3X the Proposed Maximum Use Level for a Total of Three Doses." Study Number: 2012-01-04-01-046. June 2012 to July 2012.

2. Study Location: Tulare, CA

3. Study Design:

   a. *Objective:* To evaluate the safety of pegbovigrastim when administered subcutaneously to periparturient Jersey cows and heifers at 1X (15 mg), 2X (30 mg), and 3X (45 mg) the recommended dose of 15 mg. Progeny of the treated animals were also evaluated.

   b. *Study Animals:* A total of 32 periparturient Jersey cows and heifers ranging in age from one year and seven months to eight years were used. Animals were individually housed in pens and were acclimatized within a range of seven to 24 days prior to treatment initiation. Animals were identified by individually numbered ear tags.

   c. *Treatment Groups:* Upon arrival, animals were randomly assigned to pens and once assigned to a pen, the animals stayed in that pen for the duration of the study. Cows and heifers were then randomly assigned to treatments on their anticipated day -7 prior to calving (Day 0). Four cows and four heifers were required for assignment to each of four treatment groups: control, 1X, 2X, and 3X.

      After birth, calves were housed individually in indoor pens.

Table III.A.1. Treatment Groups

Treatment Group	**Dose level**	**Dose in mg (number of syringes administered at each time point)**	**# of cows**	**# of heifers**
1	0 (control)	0 (3 saline syringes given)	4	4
2	1X	15 mg (1)	4	4
3	2X	30 mg (2)	4	4
4	3X	45 mg (3)	4	4

d. *Test Article Administration:* Pegbovigrastim was administered subcutaneously with pre-filled ready-to-use plastic syringes each containing 15 mg active ingredient. The negative control was sterile saline (0.9% NaCl), packaged in 3 mL pre-filled ready-to-use plastic syringes. Animals were administered pegbovigrastim by subcutaneous injection one, two, or three syringes in the neck when they were identified as being approximately 7 days before their anticipated calving date (Day -7), 3 days before anticipated calving date (Day -3), and within 24 hours after calving (Day 0). Three syringes of sterile saline, equivalent to the volume in the 3X dose group, were administered in the same manner and at the same time intervals to the control group.

e. *Measurements and Observations:* For cows and heifers, general health observations were performed twice daily during the duration of the study. Physical examinations were conducted within one day of arrival and on Days -7, -3, 0, and Day 4. Body weights were measured on the same days as physical examinations. Blood and urine samples were collected within two days of arrival and on Days -7, -3, 0 and Day 4 for hematology, clinical chemistry, coagulation parameters, and urinalysis. Feed and water consumption was recorded for each animal every day between Day -14 and Day 4. Milk production and milk quality (appearance) were evaluated on each animal from date of parturition to Day 4. Milk somatic cell counts were measured on Day 4. Animals were necropsied on Day 4 relative to their calving dates. Tissue samples were collected from all animals and histopathological examination was performed on all animals in all groups.

   Calves were removed from their dams after birth and fed colostrum from their dams for the first two feedings. Cow milk purchased from a local grocery store was fed to the calves after the first two feedings throughout the remainder of their observation period of 14 days. General health observations were performed twice daily and physical examinations were performed at birth, Day 7, and Day 14. Body weights were collected at the same time as physical examinations. Blood was collected on Days 7 and 14 for hematology only. Calves were not necropsied unless they died during the study.

4. Statistical Analysis: Continuous endpoints measured repeatedly were analyzed using a repeated measures analysis of covariance model with treatment, parity, day, treatment by parity, parity by day, treatment by day, and treatment by parity by day in the model as fixed effects. Baseline measurements were included as covariates. Continuous endpoints measured

once were analyzed by analysis of variance. All tests were performed at alpha=0.10. Continuous endpoints measured once were analyzed by analysis of variance with treatment, parity, and treatment by parity in the model as fixed effects. To follow up on significant effects involving dose, mean comparisons between the zero dose group and each non-zero dose group were performed (within parity, within time or overall) using linear contrasts. No adjustments were made for multiple comparisons.

5. Results: When comparing the treated groups to the control group, all treated groups showed statistically significant higher white blood cell counts; absolute neutrophil counts and percentages; absolute band cell counts and percentages; absolute metamyelocyte counts and percentages; and absolute myelocyte counts and percentages when compared to controls after initial administration of the test article on Day -7.

Table III.A.2. Follow-up comparisons of each dose group vs. control for absolute neutrophil counts (x 10^3/uL) in Margin of Safety Study 2012-01-04-01-046

Treatment Group	Least Squares Mean	P-value*
1	2.3707	
2	14.7499	0.0003
3	14.5802	0.0005
4	14.9575	0.0003

*P-value for comparing the mean of each dose level to the control

These changes were consistent with the mechanism of action of pegbovigrastim and not considered adverse. The presence of band cells and other leukocyte precursors were not considered clinically relevant and not consistently seen in all treated animals. Bone marrow evaluations did not indicate pathologic leukocyte precursor depletion in any animal.

Spleen weights were statistically significantly higher in the treated animals compared to the controls, but these differences were minor and not considered clinically relevant.

There were no cow or heifer mortalities during the study, although one animal in the 3X group was anorexic and depressed after calving and non-ambulatory by Day 4. At necropsy, this animal had mastitis, enteritis, and a perforated abomasal ulcer.

Abomasal ulcerations of varying severity were seen in some animals in all treated groups. One 1X animal had a perforated ulcer; two 2X animals each had a mild ulcer; one 3X animal had a perforated ulcer, one 3X animal had two marked ulcers, and one 3X animal had a moderate ulcer. No control animals had ulcers. Treated animals also had higher incidence rates of metritis and mastitis compared to the controls, although to a lesser degree than the ulcerations.

There were 14 calf mortalities. Six calves were stillborn or died at birth and eight calves died prior to Day 14. Of the stillbirths, two were from control animals, one from a 1X animal, and three from 2X animals. Of the eight other deaths, one was from a control animal; three from 1X animals; one

from a 2X animal; and three from 3X animals. It was concluded that high ambient temperatures (heat stress) in conjunction with protocol restrictions prohibiting the use of concomitant medications in calves contributed to this higher than normal calf mortality rate. Causes of death after birth included pneumonia, enteritis, and septicemia. These deaths are common reasons for calf mortality on a commercial dairy and were concluded to be not related to pegbovigrastim administration.

6. Conclusions: Treated animals had higher incidence rates of abomasal ulcerations/erosions, metritis, and mastitis when compared to the controls. Given the unknown incidence rates of abomasal ulcers in healthy, commercial, periparturient dairy cows for comparison to these results, it was decided a second margin of safety study should be conducted to provide independent substantiation of the findings in this study.

B. Second Margin of Safety Study

A second margin of safety study was conducted to see if the results from the first study were reproducible and to more closely study the pathology of the abomasal ulcers/gastrointestinal pathology. There were differences between the two study designs. The second study used multiparous Holsteins of a uniform body weight versus the first study which used both primiparous and multiparous Jerseys. This change was made in order to provide as uniform a study population as possible in order to remove potential confounding factors of animal size and parity as well as provide adequate statistical power and inferential value rather than lose degrees of freedom by splitting the study population into differing parities and frame sizes. The second study was also designed to reduce environmental and dietary stress on the study animals, as it was unknown how confounding these stressors were to the development of abomasal ulcers in the first study. The first margin of safety study was designed as a laboratory study where animals were re-located to the study site, had their diet changed at the study site, and were individually housed in small pens. This did not reflect actual commercial settings for dairy cows in production and may have confounded results of the first study. The second margin of safety study was designed to more closely match a routine commercial dairy. Animals in the second study remained at their home location and were individually housed in larger pens to facilitate cow comfort. Diets in the second study were unchanged because the animals remained at their home site. Additionally, while the first study conducted necropsies on Day 4 post calving, the second study conducted necropsies on Day 14 in order to observe any indications of healing if animals had ulcers. This also allowed for making more clinical observations focused on the gastrointestinal issues of the animals. Progeny of the study animals in the second margin of safety study were not evaluated because the progeny were adequately evaluated in the first margin of safety study.

1. Title: "Non-clinical Laboratory Study (GLP) to Assess Target Animal Safety in Dairy Cows Injected Subcutaneously with Pegbovigrastim at 1X, 2X, 2.5X, and 3X the Proposed Maximum Use Dose." Study Number: 2014-01-04-01-066. August 2014 to September 2014.

2. Study Location: Tulare, CA

3. Study Design:

   a. *Objective:* To evaluate the safety of pegbovigrastim when administered subcutaneously to periparturient Holstein cows at 1X (15 mg), 2X (30 mg), 2.5X (37.5 mg), and 3X (45 mg) the recommended dose of 15 mg. Progeny of the treated animals were not evaluated.

   b. *Study Animals:* A total of 45 periparturient Holstein cows ranging in age from two to three years were used. Animals were individually housed in pens and remained at their farm of origin for the study. Animals were identified by individually numbered ear tags.

   c. *Treatment Groups:* Animals were randomly assigned to pens and once assigned to a pen, the animals stayed in that pen for the duration of the study. Cows were then randomly assigned to treatments on their anticipated Day -7 prior to calving (Day 0). Nine cows were required for assignment to each of five treatment groups: control, 1X, 2X, 2.5X, and 3X.

      After birth, calves were returned to the commercial herd.

Table III.B.1. Treatment Groups

Treatment Group	**Dose level**	**Dose in mg (number of syringes administered at each time point)**	**# of cows**
1	0 (control)	0 (3 saline syringes given)	9
2	1X	15 mg (1)	9
3	2X	30 mg (2)	9
4	2.5X	37.5 mg (2.5)	9
5	3X	45 mg (3)	9

   d. *Test Article Administration:* Pegbovigrastim was administered subcutaneously with pre-filled ready-to-use plastic syringes each containing 15 mg pegbovigrastim. The negative control was sterile saline (0.9% NaCl), packaged in 3 mL pre-filled ready-to-use plastic syringes. Animals were administered pegbovigrastim by subcutaneous injection of one, two, two and a half, or three syringes in the neck when they were identified as being approximately 7 days before their anticipated calving date (Day -7) and within 24 hours after calving (Day 0). Three syringes of sterile saline equaling the volume of the 3X group were administered in the same manner and at the same time intervals to the control group.

   e. *Measurements and Observations:* General health observations were performed twice daily during the duration of the study. Physical examinations were conducted on Days -21, -7, 0, 7, and Day 14. Blood samples were collected on the same days as physical examinations for hematology (including reticulocyte counts), clinical chemistry, and fibrinogen. Body weights and feed consumption were recorded for each animal. Milk production and milk quality (appearance) were evaluated on each animal from date of parturition to Day 14. Milk somatic cell counts were measured on Days 4, 7, 10 and 13. Fecal occult blood was measured on each animal daily from Day -20 until the end of the study on Day 14. Animals were necropsied on Day 14 relative to their calving

dates. The following tissues were examined for gross lesions at necropsy: abdominal cavity, gastrointestinal tract from esophagus to large intestine, uterus, and mammary tissue. Representative tissue samples were collected from these tissues in all animals and histopathological examination was performed on all animals in all groups.

4. Statistical Analysis: Continuous endpoints measured repeatedly were analyzed using a repeated measures analysis of covariance model with treatment, time, and treatment by time in the model as fixed effects. Baseline measurements were included as covariates. All statistical comparisons were performed at alpha=0.10. Continuous endpoints measured once were analyzed by analysis of variance with treatment as a fixed effect. To follow up on significant effects involving dose, mean comparisons between the zero dose group and each non-zero dose group were performed (within time or overall) using linear contrasts. No adjustments were made for multiple comparisons.

5. Results: When comparing the treated groups to the control group, all treated groups showed statistically significant higher white blood cell counts; absolute neutrophil counts and percentages; and absolute band cell counts and percentages when compared to controls after initial administration of the test article on Day -7.

Table III.B.2. Follow-up comparisons of each dose group vs. control for absolute neutrophil counts (x 10^3/uL) at each time point in Margin of Safety Study 2014-01-04-01-066

Study Day	Treatment Group	Least Squares Mean	P-value*
0	1	5.7244	
0	2	9.6639	0.1969
0	3	16.3643	0.0011
0	4	14.8478	0.0043
0	5	17.0915	0.0005
7	1	3.8456	
7	2	10.1029	0.0191
7	3	16.2360	<0.0001
7	4	11.3743	0.0058
7	5	17.5452	<0.0001
14	1	3.1870	
14	2	6.5544	0.0612
14	3	8.9725	0.0027
14	4	6.7804	0.0499
14	5	7.8329	0.0112

*P-value for comparing the mean of each dose level to the control

These hematology changes were consistent with the mechanism of action of pegbovigrastim and not considered adverse. The presence of band cells was not considered clinically relevant and not consistently seen in all treated animals. Bands in all treated groups except for the 3X group were gone by Day 14.

Reticulocyte counts were variable across all treated and control groups and not considered clinically relevant.

There were three positive fecal occult blood results during the study: two in control animals and one in a 1X animal. These were considered sporadic and clinically insignificant. There were no reports of tarry or bloody stool in any animal during the study.

There were no clinically significant differences between treated and control animals in physical examinations, milk production, SCCs, body weights, or feed consumption. Feed intake trends were similar across all treatment groups with a decrease around calving and a gradual increase over the subsequent two weeks. There were no cow mortalities during the study.

On necropsy, ulcers, if present, were classified grossly by the following scale:

Type 0: An erosion
Type I: An ulcer without hemorrhage
Type II: An ulcer with hemorrhage
Type III: A perforated ulcer with acute localized peritonitis
Type IV: A perforated ulcer with acute diffuse peritonitis
Type V: A perforated ulcer with peritonitis within the omental bursa

On histopathologic examination, any ulcer was also graded as follows:

Grade 1 (minimal): Necrosis only extends to or into the muscularis mucosa
Grade 2 (mild): Necrosis extends through the muscularis mucosa and into the submucosa, but not into the deeper circular or longitudinal muscle layers (muscularis externa)
Grade 3 (moderate): Necrosis extends through the muscularis mucosa, through the submucosa, and into the muscularis externa
Grade 4 (marked): Ulcer extends through the muscularis mucosa, through the submucosa, through the muscularis externa and through the serosa (perforation)

On necropsy and at histopathologic examination, no study animals had abomasal ulcers; however, treated animals had higher incidence rates of abomasal erosions and reddened mucosa in various parts of the lower gastrointestinal tract as compared to control animals. These changes were considered test-article related but not clinically relevant due to their mild nature. On histopathology of these erosions, acute and mild inflammation was most commonly reported.

Adverse Events: Four sets of twins were born during the study and three of these sets were stillborn: two sets in the 3X group and one set in the 2.5X group. Necropsies determined causes were likely placental insufficiency. These stillborns were not considered test article related. The surviving set of twins was born to an animal in the 2.5X group.

6. Conclusions: Pegbovigrastim administered subcutaneously to periparturient dairy cows at approximately seven days prior to calving and within 24 hours after calving resulted in a higher incidence rate of abomasal erosions when compared to the controls. These differences were considered not clinically relevant as there were no clinical signs observed in the animals.

C. Injection Site Safety Study

1. Title: "Injection Site Toleration of PEG bG-CSF Administered by Subcutaneous Injection to Dairy Cows." Study Number: 2012-01-04-01-044. March 2012 to April 2012.

2. Study Location: Tulare, CA

3. Study Design:

   a. *Objective:* To evaluate the injection site toleration of non-lactating cows treated subcutaneously with one injection (15 mg) of pegbovigrastim either 14 days prior to necropsy or 12 hours prior to necropsy.

   b. *Study Animals:* 12 open, non-lactating, multiparous Holstein cows were used in this study. Cows ranged from two to seven years of age. All animals were individually housed in dry lot pens. Animals were identified by individually numbered ear tags and were acclimated for 14 days prior to study initiation.

   c. *Treatment groups:* Six animals were randomly assigned to one of two treatment groups. Each group received one injection (15 mg). Treatment Group One received treatment on Day 0 and was necropsied 14 days later. Treatment Group Two received treatment on Day 13 and was necropsied 12 hours later. The experimental unit was the individual animal. There was no control group.

   d. *Test Article Administration:* Pegbovigrastim was administered subcutaneously with a pre-filled ready-to-use plastic syringe containing 15 mg active ingredient. Animals were administered pegbovigrastim by subcutaneous injection in the neck at either 14 days prior to necropsy or 12 hours prior to necropsy.

   e. *Measurements and Observations:* Injection sites were evaluated once daily beginning on Day 0 prior to injection and immediately prior to euthanasia. General behavioral observations including lameness and sensitivity were also performed. Injection sites were observed for abnormalities including:

      1) Skin appearance (normal erosions, ulcerations, or abscesses)
      2) Swelling (length, width, and height) measured to the nearest millimeter using a caliper
      3) Heat (presence or absence on palpation)
      4) Redness (if skin pigmentation permits)
      5) Texture (normal, soft, or firm on palpation)

      All animals were necropsied and were limited to a complete gross evaluation of the injection sites. Gross pathology assessments were divided into four levels of examination:

      1) The external skin surface over the injection site
      2) The subcutaneous tissue
      3) The surface of the neck musculature at the injection site
      4) The deep interior of the neck musculature at the injection site

Color photographs were taken of each injection site for each of the four levels of gross examination. The entire side of the neck where the injection was administered was prosected. If no lesions were grossly visible, samples were not collected for histopathological assessment.

4. Statistical Analysis: There was no statistical analysis for this study.

5. Results: There were no mortalities during the study. There were no abnormal findings on physical examinations. Test article related injection site daily observation abnormalities were limited to injection site swellings about twelve hours after test article administration in five animals (2, 8, 9, 12, and 15), all of which were in Treatment Group 2 which was dosed approximately 12 hours prior to necropsy. The swellings ranged from 40 to 50 mm long, 25 to 30 mm wide, and 5 mm high. All swellings had normal texture and no heat or redness was noted. Histopathologically, injection sites with grossly visible reactions were characterized by mild acute multifocal subcutaneous inflammation with minimal to mild hemorrhage in two of the five affected animals. In four cases where the underlying muscle was sectioned there was minimal to mild multifocal acute inflammation in the superficial neck muscle beneath the subcutaneous reaction.

   Adverse Events: There were no adverse events during the conduct of this study.

6. Conclusions: It was concluded that this product would not require carcass trim at slaughter, therefore a trim loss statement is not required on the labeling.

D. Reproductive Safety

Reproductive safety was evaluated as part of the effectiveness field study. Cow health, gestation length, live birth rate, neonatal calf health, and first service conception rates were measured across five sites that included a total of 243 heifers and 558 cows (801 animals total). Both small frame (Jerseys) and large frame (Holstein and Holstein-cross) breeds were enrolled in the study. Animals were administered either 15 mg pegbovigrastim or 3 mL saline by subcutaneous injection in the neck approximately seven days prior to their anticipated calving date and again within 24 hours after calving.

1. Health Observations and Mortality: 28 adult animals died during the course of the study. Mortalities were attributable to conditions routinely seen in commercial dairy herds, including toxic mastitis, peritonitis, and bronchopneumonia. No deaths were attributable to pegbovigrastim and there was no clinical or statistically significant difference in the mortality rates between treated (3%) and control (4%) groups ($p = 0.5835$).

2. Gestation length: There was no statistically significant difference between the treated (7.5 ±0.6 days) and control (7.7 ±0.6 days) group from the day the first injection was administered (estimated seven days prior to calving) to the day of calving ($p = 0.6057$).

3. Percent live births: There was no statistically significant difference between the treated (94.9%) and control (96.4%) group in the percentage of live births across the five sites (p = 0.2919).

4. Calf health observations and mortality: Abnormal health observations and mortality in calves in this study were at acceptable rates of disease and loss, and were attributable to common calf diseases within the commercial dairy industry, namely scours and pneumonia. The Wisconsin site experienced relatively high calf mortality due to a severe outbreak of scours which equally affected the treated and control groups. There were no statistically significant differences in mortality between calves born to dams in the treated (13.2%) and control (10.3%) groups across the five sites (p = 0.1794).

5. First service conception rates: There was no statistically significant difference between the treated (42.2%) and control (36.7%) group in first service conception rates across the five sites (p = 0.3520).

Conclusions: There were no test article related changes observed regarding reproductive safety.

E. Assessment of Particulates

Subvisible particulates composed of pegbovigrastim and lubricant, inherent to the product and syringe formulations, were present in the batches of syringes used in the target animal safety and effectiveness studies conducted in the United States. Batches containing small numbers of visible pegbovigrastim/lubricant particulates were present along with subvisible particulates during process validation. It was determined through review of scientific literature on protein particulates and immunogenicity and chemistry data of the product, in conjunction with the clinical features of this product as evaluated during pre-approval studies conducted both in the U.S. and in foreign countries, that neither visible nor subvisible particulates of this nature had an effect on animal safety or effectiveness of the product. A few visible small, translucent or white particles may be present in this product. A syringe should not be shaken prior to use in order to allow for observation of the presence of particulates as compared to air bubbles. This product should not be used if it is discolored or cloudy or if other particulate matter is present.

F. Label statement about adverse effects noted from foreign studies and abomasal ulcerations/erosions.

ADVERSE REACTIONS: Some cases of hypersensitivity-type reactions have been observed in studies outside the United States within five minutes to two hours, occurring most often after the first administration of Imrestor. Clinical signs may include elevated respiratory rate, dyspnea, urticaria, sweating, dependent edema, swollen mucous membranes, and/or hypersalivation, and, rarely, death. These reactions resolve within hours of onset with or without therapeutic intervention and have not been shown to reoccur with subsequent injections of Imrestor.

Abomasal ulcerations/erosions were observed in the Margin of Safety studies. (See Target Animal Safety Section.)

IV. HUMAN FOOD SAFETY

A. Antimicrobial Resistance

PEGylated recombinant bovine granulocyte colony stimulating factor (PEG bG-CSF) is not considered to be an antimicrobial compound, or reported to impact antimicrobial resistance among bacteria of public health concern in or on treated periparturient dairy cows and periparturient replacement dairy heifers. The Agency determined that an assessment of the antimicrobial resistance associated with this use of PEG bG-CSF in periparturient dairy cows and periparturient replacement dairy heifers is not necessary at this time.

B. Impact of Residues on Human Intestinal Flora

PEG bG-CSF is not considered to be a typical antimicrobial compound. Literature available in the public domain, as well as data from standard limiting dilution MIC assays against multiple isolates of *Staphylococcus aureus*, *Streptococcus uberis*, *Escherichia coli*, *Actinobacillus pleuropneumoniae*, *Mannheimia haemolytica*, *Pasteurella multocida*, *Salmonella choleraesuis*, *Salmonella typhimurium*, and *Histophilus somni*, performed according to Clinical Laboratory Standards Institute guidelines, indicate that PEG bG-CSF does not exert direct antimicrobial effects on bacteria. Further, residues of PEG bG-CSF in or on edible tissues or food products from treated periparturient dairy cows and replacement dairy heifers are not predicted and have not been reported to impact the intestinal flora of human consumers. The Agency determined that an assessment of the impact of residues of PEG bG-CSF on human intestinal flora and establishment of a microbiological acceptable daily intake are not necessary at this time.

C. Toxicology and Residue Chemistry

The Agency has determined that the traditional paradigm of addressing the toxicology and residue chemistry components of the Human Food Safety technical section is not the most appropriate approach to evaluate the safety to humans from consuming IMRESTOR (pegbovigrastim injection) residues. No traditional toxicology and residue chemistry studies were conducted to support this approval. The human food safety of IMRESTOR has been evaluated based on 1) the hazard identification and characterization of the pegbovigrastim injection residues, which includes the PEG, protein and its metabolites; and 2) an assessment of human exposure to the pegbovigrastim injection residues.

1. *IMRESTOR Residues* - The IMRESTOR product contains PEGylated recombinant bovine granulocyte colony stimulating factor (PEG bG-CSF) in a buffered sodium acetate solution. The sequence of bG-CSF corresponds to the 175 amino acid sequence [GenBank Accession Number AAD16102] of the endogenously produced bG-CSF cytokine with the exception of the amino terminal methionine and a single amino acid substitution, whereby a non-genetically encoded amino acid is incorporated into the protein to enable a site specific covalent attachment of a 20-kDa polyethylene glycol (PEG) molecule to the protein.

2. The Agency does not have human food safety concerns for bG-CSF that is already naturally present in meat, nor the individual components (amino acids) of proteins, as these components are present in various foods

consumed by humans, and there is no known inherent risk in consuming these foods. PEG (mean molecular weight 200-9,500) is approved for use as a direct food additive under the provisions of Section 172.820 of Title 21 of the Code of Federal Regulations. The PEG component, as well as the buffering agents, pose no human food safety concern under the conditions of use.

Sequence Analysis of pegbovigrastim injection – The protein sequence homology of bG-CSF with known human allergenic proteins was analyzed. Based on the analysis, no biologically meaningful homologs of known human allergens for both linear and conformational IgE epitopes are expected to occur. Therefore, the Agency concludes that there is little immunogenic or allergenic response concern for residues of pegbovigrastim injection in edible tissues.

3. Human Food Safety Studies

   Studies used to determine the human food safety of IMRESTOR (pegbovigrastim injection) are summarized below:

   a. *In Vitro* Stability Study in Simulated Gastric Fluid (Size Exclusion Chromatography)

      1) Study Title: PEG-bGCSF Drug Product Degradation Studies in Simulated Gastric Fluid (SGF) by Size Exclusion Chromatography

      2) Study Number: DBA13-003

      3) Report Number: RPT23337.00

      4) Report Date: November 17, 2011

      5) Performing Laboratory (in-life) Location: Wilmington, North Carolina

      6) Experimental Design: A pepsin stability study was conducted to test the stability of pegbovigrastim injection in simulated gastric fluid that mimics the conditions of the human gastric environment. The study was conducted using a modified protocol for evaluating the potential allergenicity of proteins (Regulatory Toxicology and Pharmacology (2004) 39: 87-98). Briefly, the test article, PEGylated Bovine Granulocyte Colony Stimulating Factor (PEG bG-CSF) at 2.0 mg/mL in simulated gastric fluid (SGF) (sodium chloride (0.2% w/v) in hydrochloric acid (0.7% v/v) pH 1.0-1.4 from RICCA Chemical Company) was incubated for 0, 0.5, 2, 4, and 8 hours at 37 °C. Pepsin (3.2 g/L) and deionized water were added prior to the experiment. Samples were quenched with 1 N sodium hydroxide at pH 5.1-8.3 (see Table IV.C.1). Control samples contained the SGF and pepsin alone. The samples were analyzed by size-exclusion chromatography for determination of intact protein.

Table IV.C.1. Samples

Sample Number	Incubation Time (hours)	Treatment	pH
1	0	Control	6.0
2	8	Control	7.5
3	0	PEG bG-CSF	7.2
4	0.5	PEG bG-CSF	8.3
5	1	PEG bG-CSF	5.9
6	2	PEG bG-CSF	5.2
7	4	PEG bG-CSF	5.1
8	8	PEG bG-CSF	7.5

7) Results and Conclusion: Chromatograms demonstrated the ability of the method to separate the PEG bG-CSF monomer, dimers and polymeric aggregates. Peak area was visualized by UV at 214 nm and compared to a reference standard. Sample retention times and peak areas from seven peaks were identified. The control samples of SGF with pepsin in the absence of protein (samples 1 and 2) indicated two main peaks at retention times of 6.4 minutes and 7.6 minutes. PEG bG-CSF incubated at zero minutes with PEG bG-CSF (sample 3) resulted in two major chromatographic peaks at retention times of 7.2 minutes to 8.3 minutes. Following incubation with SGF and pepsin (samples 4-8), the intact PEG bG-CSF was not detected within 30 minutes at 37 °C; however, peak areas were detected at retention times of 8.5 minutes. The high instability of the intact protein under the assay conditions suggests that PEG bG-CSF is likely not to remain intact in the human gastrointestinal tract.

b. *In Vitro* Stability Study in Simulated Gastric Fluid (Reversed Phase Chromatography)

1) Study Title: PEG-bGCSF Drug Product Degradation Studies in Simulated Gastric Fluid (SGF) by Reversed Phase Chromatography

2) Study Number: DBA13-004

3) Report Number: RPT23338.00

4) Report Date: November 17, 2011

5) Performing Laboratory (in-life) Location: Wilmington, North Carolina

6) Experimental Design: A second pepsin stability study was conducted to test the stability of pegbovigrastim injection in simulated gastric fluid that mimics the conditions of the human gastric environment. The study was conducted using a modified protocol for evaluating the potential allergenicity of proteins (Regulatory Toxicology and Pharmacology (2004) 39: 87-98). Briefly, the test article, PEG bG-CSF at 2.0 mg/mL in simulated gastric fluid (SGF) (sodium chloride (0.2% w/v) in hydrochloric acid (0.7% v/v) pH 1.0-1.4 from RICCA Chemical Company) was incubated for 0, 0.5, 2, 4, and 8 hours at 37 °C. Pepsin (3.2 g/L) and deionized water were added prior to the experiment. Samples were quenched with 1 N sodium hydroxide at

pH 5.1-8.3 (see Table IV.C.2). Control samples contained the SGF and pepsin alone. The samples were analyzed by reversed phase HPLC for determination of intact protein.

Table IV.C.2. Samples

Sample Number	Incubation Time (hours)	Treatment	pH
1	0	Control	6.0
2	8	Control	7.5
3	0	PEG bG-CSF	7.2
4	0.5	PEG bG-CSF	8.3
5	1	PEG bG-CSF	5.9
6	2	PEG bG-CSF	5.2
7	4	PEG bG-CSF	5.1
8	8	PEG bG-CSF	7.5

7) Results and Conclusion: Chromatograms demonstrated the ability of the method to detect PEG bG-CSF and bG-CSF from impurities and degradation products. Peak area was visualized by UV at 214 nm then compared to a reference standard. Control samples in the absence of protein (samples 1 and 2) showed no protein peak. PEG bG-CSF incubated at zero minutes with PEG bG-CSF (sample 3) resulted in 10 chromatographic peaks at retention times of 17.5 minutes to 21.2 minutes. Sample retention times were only detected at 8.7 to 9.0 minutes following incubation with SGF and pepsin (samples 4-8) while the chromatographic peaks at 17.5 minutes to 21.2 minutes were no longer detected. The intact PEG bG-CSF protein was not detected within 30 minutes incubation at 37 °C. The high instability of the protein in the assay conditions suggests that PEG bG-CSF is not likely to remain intact in the human gastric environment.

c. Bioavailability Study in Rats

1) Study Title: Bioavailability of PEG bG-CSF to Rats Following Subcutaneous and Oral Dosing

2) Study Number: WIL-668009

3) Study Dates: September, 2010 – January, 2011

4) Performing Laboratory (in-life) Location: Ashland, Ohio

5) Experimental Design: The objective of this study was to determine the bioavailability of TI33 20K PEG bG-CSF following subcutaneous and oral administration to rats. The test article, PEG bG-CSF, was a frozen liquid formulation (approximately 2 mg/mL), and thawed immediately prior to use. Sprague Dawley rats, approximately 10 - 12 weeks of age and weighing 240 to 351 g at dosing, were used. Following an acclimation period of at least 10 days, 18 rats (9 males and 9 females) were assigned to one of three groups and treated with either a single dose of formulation buffer or PEG bG-CSF, as described in the table below.

Table IV.C.3. Treatment Regimen

Group Number	Number of Animals	Treatment	Dose Level (µg/kg bw)	Route of Administration
1	3 males and 3 females	Control	NA*	Subcutaneous
2	3 males and 3 females	PEG bG-CSF	250	Subcutaneous
3	3 males and 3 females	PEG bG-CSF	2500	Oral

*not applicable

Blood samples were collected from each animal at pre-dose, and 4, 13, 24, 48, 72, 96 and 120 hours post dosing. Endogenous granulocyte colony stimulating factor stimulates the survival, proliferation, differentiation, and function of neutrophil precursors and mature neutrophils. Therefore, a portion of the sample was used to obtain absolute neutrophil counts, while the remaining portion was processed to serum to determine PEG bG-CSF levels. Absolute neutrophil counts were determined using a Bayer ADVIA 120 Hematology Analyzer. Serum samples were stored frozen and shipped on dry ice to Midwest BioResearch for PEG bG-CSF analysis. PEG bG-CSF in serum was analyzed using a validated immunoassay procedure: PBA08-147; Bioanalytical Procedure of an Electrochemiluminescent (ECL) Immunoassay for the Quantification of PEG bG-CSF in rat serum. The assay had a quantification range from 46.9 to 6,000 ng/mL.

6) Results and Conclusion:

Table IV.C.4. Mean Neutrophil Concentration (thousands/µL; ± Standard Deviation) in Rats

Group Number	Pre-Dose	4 hours	12 hours	24 hours	48 hours	72 hours	96 hours	120 hours
1	3.14 ± 0.56	4.15 ± 0.90	3.19 ± 0.99	4.51 ± 1.21	5.81 ± 2.02	5.20 ± 1.46	5.15 ± 1.02	5.05 ± 1.10
2	2.41 ± 0.60	5.63 ± 0.80	15.18 ± 3.24	26.86 ± 6.8	29.39 ± 6.06	22.17 ± 6.28	11.19 ± 4.20	7.88 ± 3.03
3	2.04 ± 0.61	3.53 ± 1.96	3.14 ± 0.76	5.75 ± 3.30	5.38 ± 1.29	3.81 ± 1.21	3.87 ± 1.41	3.85 ± 1.02

Serum PEG bG-CSF concentrations above the LOQ of 46.9 ng/mL were only detected in animals in Group 2 (SC treatment with PEG bG-CSF) at the 4 - 72 hour time points; all other time points were below the LOQ. A summary of the concentrations in Group 2 is shown in Table IV.C.5 below.

Table IV.C.5. PEG bG-CSF Concentration (ng/mL, mean ± Standard Deviation) in Serum of Rats Following Subcutaneous Administration of 250 µg/kg PEG bG-CSF

Group	4 hours	12 hours	24 hours	48 hours	72 hours
2	511 ± 425	11781 ± 4225	15086 ± 3583	3238 ± 1005	374 ± 232

The above study indicates that PEG bG-CSF is not bioavailable after oral administration in rats.

4. Overall Conclusion

A worst-case scenario estimates that the maximum human exposure to pegbovigrastim residues in edible tissue (injection site) and milk would be 0.5 mg/kg BW and 0.025 mg/kg BW, respectively. The scenario in edible tissues is based on the proposed use of PEG bG-CSF, if a person consumed the entire injection site following two injections (30 mg) in a single service of meat for a 60 kg person resulting in an exposure of 0.5 mg/kg BW. We considered a second scenario in which the entire two doses were in the milk of a typical Holstein Friesian cow (average milk production 31 L on the third day of lactation). Under this scenario, the concentration in milk would be 30 mg/31 L or 1.0 mg/L. Therefore, the total daily exposure would be (1 mg/L x 1.5 L/person)/60 kg BW, which is equal to 0.025 mg/kg BW. At concentrations well above these worst-case exposure estimates, the *in vitro* and *in vivo* studies support that pegbovigrastim injection will have very low oral bioavailability and lack intact protein stability in humans consuming edible tissues from IMRESTOR–treated periparturient dairy cows and periparturient replacement dairy heifers. The Agency therefore does not expect any meaningful biological effects of potential pegbovigrastim injection residues on the human consumer.

The Agency does not have human food safety concerns for bG-CSF or the individual amino acids of this IMRESTOR (pegbovigrastim injection), as these components are essential amino acids to humans, are present in various foods consumed by humans, and there is no known inherent risk in consuming these foods. The Agency has concluded that there are no human food safety concerns for the use of IMRESTOR (pegbovigrastim injection) in periparturient dairy cows and periparturient replacement dairy heifers.

D. Human Food Safety Parameters

1. Establishment of the Final Acceptable Daily Intake (ADI) or Acute Reference Dose (ARfD)

   An ADI or an ARfD is not needed.

2. Safe Concentrations for Total Residues (Edible Tissues and Injection Sites)

   Safe concentrations for total residues of IMRESTOR (pegbovigrastim injection) in edible tissues or injections sites of periparturient dairy cows and periparturient replacement dairy heifers are not needed.

3. Target Tissue and Marker Residue

   It is not necessary to assign a target tissue or a marker residue for IMRESTOR (pegbovigrastim injection) residues in periparturient dairy cows and periparturient replacement dairy heifers.

4. Tolerances

   Tolerances for IMRESTOR (pegbovigrastim injection) in edible tissues are not required.

5. Withdrawal Period and Milk Discard Time

   The approved use of IMRESTOR (pegbovigrastim injection) in periparturient dairy cows and periparturient replacement dairy heifers does not require a withdrawal period (*i.e.*, zero withdrawal) or a milk discard time (*i.e.*, zero milk discard).

E. Analytical Method for Residues

A regulatory method for IMRESTOR (pegbovigrastim injection) is not required.

V. USER SAFETY

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to IMRESTOR:

Not for use in humans. Keep out of reach of children.

In case of accidental self-injection, wash the site of injection thoroughly with clean running water. Foreign proteins such as pegbovigrastim have the potential to cause anaphylactic-type reactions. If you experience swelling or redness at the site of exposure, or more severe reactions such as shortness of breath, seek medical attention immediately and take the package insert with you. Report the event to Elanco Animal Health at 1-800-428-4441. To obtain a Safety Data Sheet, contact Elanco Animal Health at 1-800-428-4441.

VI. AGENCY CONCLUSIONS

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act (FD&C Act) and 21 CFR part 514. The data demonstrate that IMRESTOR, when used according to the label, is safe and effective for the reduction in the incidence of clinical mastitis in the first 30 days of lactation in periparturient dairy cows and periparturient replacement dairy heifers. Additionally, data demonstrate that residues in food products derived from species treated with IMRESTOR will not represent a public health concern when the product is used according to the label.

A. Marketing Status

Labeling restricts this drug to use by or on the order of a licensed veterinarian. This decision was based on the following factors: 1) adequate directions cannot be written to enable lay persons to appropriately diagnose and subsequently use this product for reduction in the incidence of clinical mastitis; and 2) professional

expertise is required to monitor the safe use of the product, including treatment of any adverse reactions.

B. Exclusivity

IMRESTOR, as approved in our approval letter, qualifies for FIVE years of marketing exclusivity beginning as of the date of our approval letter. This drug qualifies for exclusivity under section 512(c)(2)(F)(i) of the FD&C Act because this is the first time we are approving this active ingredient in a new animal drug application submitted under section 512(b)(1) of the FD&C Act.

C. Patent Information:

For current information on patents, see the Animal Drugs @ FDA database or the Green Book on the FDA CVM internet website.